CYTOARCHITECTURE OF ECDYSIAL GLAND/Y-ORGAN OF *CHARYBDIS ANNULATA* (FABRICIUS) (CRUSTACEA: BRACHYURA)

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ABSTRACT

The cytomorphology and cytochemistry of the ecdysial gland/Y-organ in a male brachyuran crab, *Charybdis annulata*, are examined. These organs are opalescent, lobulated, epithelioid structures embedded in brown fatty tissue. Lobules are interconnected with haemal sinuses and capillaries. The cell boundaries are indistinct and mitotic figures are absent. Cytochemically, the lobules of the Y-organ are faintly positive to Schiff's alone and alcian blue staining. These lobules are rich in proteins with amino and sulfhydryl groups, as well as in lipids and phospholipids. The pyrinophilic nature of the organ has also been noticed.

INTRODUCTION

THE crustacean ecdysial gland/Y-organ present in the cephalothorax controls moulting, reproduction and other physiological activities. Subsequent to its discovery in the malacostracans by Gabe[1,2] the Y-organ has been the subject of considerable research, which has led to the establishment of the synthesis of moulting hormone or ecdysone in it[3].

The role of the Y-organ in moulting is well established. Investigations have been carried out on the histomorphological variations that occur in the Y-organ during the moult cycle in various decapods[4–7]. Changes in the histological profile of the Y-organ during different phases of gonad maturation have also been studied[8,9]. Although no definite data exist concerning the cytochemical nature of the crustacean Y-organ, crustacean ecdysteroids have been chemically defined, and their site of synthesis, mode of transport and effect upon a number of physiological parameters have been explored. The paucity of information on the cytochemistry of the Y-organ led us to investigate this question in *C. annulata*.

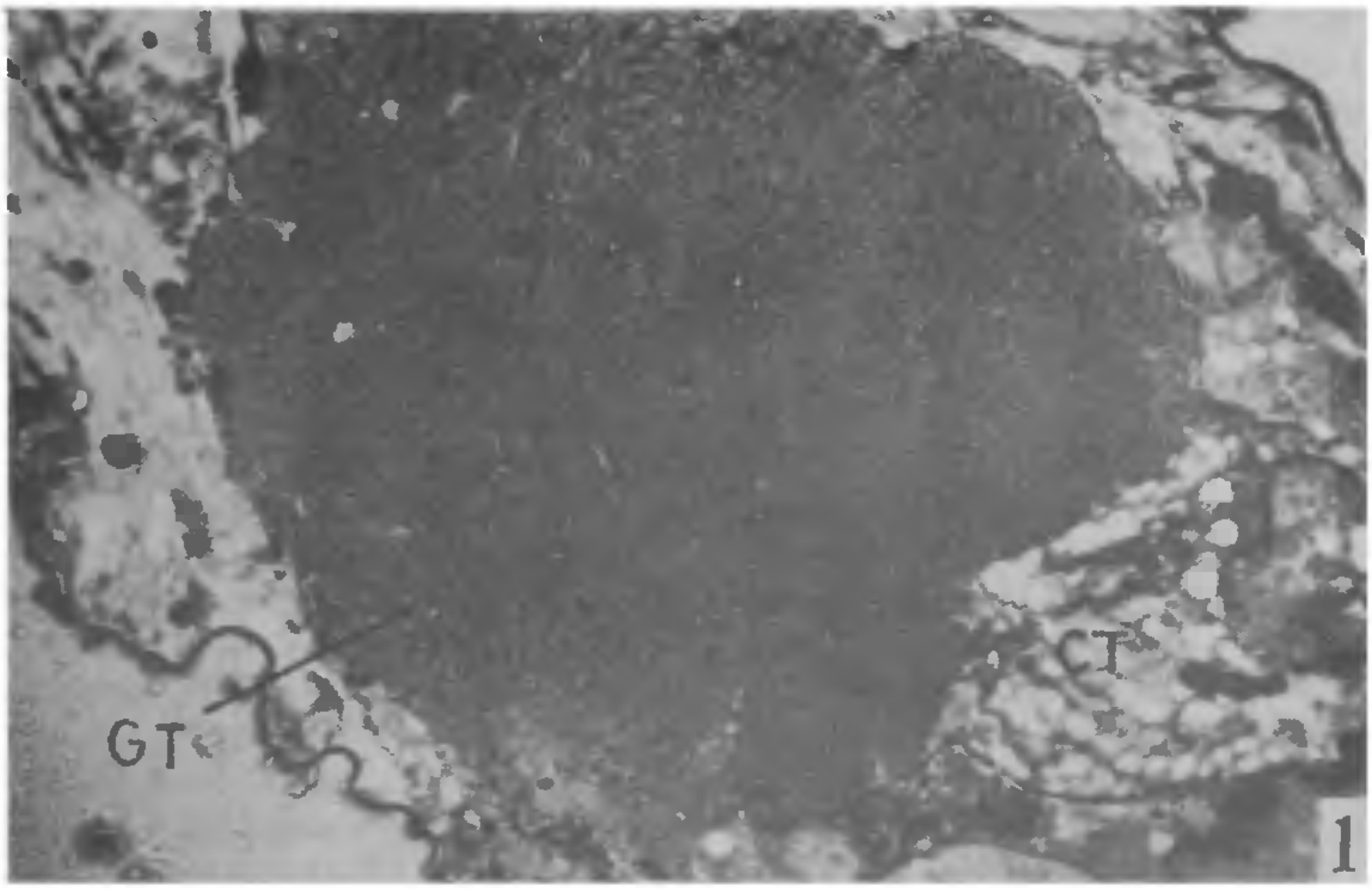
MATERIALS AND METHODS

Live male *C. annulata* were obtained from an offshore fishing station in the vicinity of Visakhapatnam. For the present study maturing crabs were considered. The carapace was removed and the Y-organs were excised very quickly well before the haemolymph formed a clot around them and were fixed in Susa, Zenkers, formal calcium and Carnoy solutions. After the usual procedure of dehydration and infiltration, sections of 8μm thickness were cut. The general cytological stains used and various cytochemical techniques employed to study polysaccharides, proteins, lipids and nucleic acids were adopted from the standard procedures of Pearse[10] and Bancroft[11].

RESULTS

The Y-organ of *C. annulata* is a paired, conical glandular epidermal structure, located in the cephalothorax anterior to the branchial chamber just posterior and immediately lateral to the eyestalks. The glands are in intimate contact with connective and lymphogenous tissue and are directly applied to the hypodermis of the ventral carapace at the intersection of the two skeletal ridges. In a live specimen the glands can be readily distinguished from surrounding material (hypodermis, haemal sinus and muscle) by their pale yellow translucent appearance. Sometimes the glandular haemocytes are found in close association with the glands. In crabs of 9 cm carapace width the distance between the two glands is approximately 4.2 cm, and the gland measures 1 mm long and 0.75 mm wide. The glands are constantly bathed in haemolymph because they lie in a shallow, scooped-out epidermal depression filled with haemal fluid. They have neither a lumen nor a secretory canal. No nerve supply could be traced within the organ, even after very careful observations.

Cytologically the Y-organ (figures 1 and 2) consists of anastamosing lobules of epithelial cells



Figures 1–2. Intermoult Y-organ. **1.** Section through Y-organ of *Charybdis annulata*; [CT, Connective tissue; GT, Glandular tissue]. **2.** Portion enlarged, showing the arrangement of lobules [H, Haemocyte; VT, Vascular tissue].

separated by numerous interconnected haemal sinuses. Each lobule is enveloped by a thin layer of connective tissue. The interlobular areas of the glands are loose. Frequently, haemocytes, vascular tissue and supporting cells are observed between the free edges of two adjacent lobules. Each lobule consists of ten to twenty cells. The cellular limits are not quite clear. The cells have roughly spherical nuclei approximately 4–5 μm in diameter and the nucleus is often acentric and contains one or more peripherally or centrally located nucleoli with peripherally condensed chromatin. Mitotic activity is

not evident in any of the cells of the organ. The cytoplasm of the cells can be quite variable, ranging from very homogeneously staining with Heidenhain's Azan stain, to highly vacuolated. Cell degeneration or necrosis in the Y-organ was not observed in the present study.

To study the chemical characteristics of the Y-organ a series of cytochemical tests have been done on sections of the organ. The results are presented in table 1.

The lobules of the Y-organ give a moderate PAS-positive reaction resistant to diastase digestion but labile to acetylation. The lobules are faintly positive to Schiff's and alcian blue staining. They are strongly mercuric bromophenol blue positive, react negatively to *p*-dimethylaminobenzaldehyde nitrite, but respond slightly to Millon's reagent. They stain faintly with potassium permanganate/alcian blue, strongly with ferric ferricyanide and Congo red, but intensely with ninhydrin/Schiff. In the pyronin Y and Feulgen reactions they are positive. The lobules are moderately positive to copper phthalocyanin and Sudan black B owing to the presence of phospholipids and lipids.

DISCUSSION

The general anatomical description of the Y-organ of *C. annulata* is identical with that of other brachyurans. The gland is oval in shape and is situated in the facial region, ventrolateral to the eye socket between the mandibular external adductor muscle and the adjacent branchiostegite at the anterolateral edge of the branchial chamber. The Y-organ consists of anastomosing lobules of epithelial cells separated by interconnected haemal sinuses and capillaries. No nerve supply was traced within the Y-organ. This is in agreement with Gabe's[2] observation in the crab, *Carcinus*, wherein he concluded that the neurosecretory material was not transported intra-axonally to the Y-organ. But in Echalier's[12] observations on the same species the Y-organ was said to be innervated by a nerve that originates in the suboesophageal ganglion (commissural ganglion?) and capillaries arising from a branch of the antennary artery penetrate it. However, Passano[13] stated that the nerves merely passed by the Y-organ, and Le Roux[5] was unable to find any nerves supplying the Y-organ of *Palaemon serratus*. Conceivably the Y-organ is controlled by neuroendocrine signals transmitted through vascular channels[14].

The lobules are made up of many small cells often with indistinct outlines (cell boundaries) and without any aldehyde fuchsin positive secretory material within the cytoplasm. These observations differ from those of Durand[15] on *Orconectes limosa*, where the cell boundaries and aldehyde fuchsin positive granules are distinct. Madhyastha & Rangneker[16], and Hussain & Vasantha[9] described two cell types in the lobules of the Y-organ. The more abundant cell type

Table 1 *Cytochemical tests done on sections of Y-organ of C. annulata*

Cytochemical test	Reacting substances	Results
Periodic acid/Schiff (PAS)	Carbohydrates	+
PAS/saliva	Glycogen	±
PAS/acetylation	1:2 glycol groups	−
PAS/deacetylation	1:2 glycol groups	±
Schiff's	Free aldehyde groups	±
Alcian blue (AB) pH 1	Sulfated mucosubstances	±
AB pH 2.5	Acid mucosubstances	±
Aldehyde fuchsin (AF)	Sulfated mucosubstances	−
Bromophenol blue (BPB)	Basic proteins	+ +
Van Slyke's/BPB	Basic proteins	−
Millon's reaction	Tyrosine	±
p-dimethylaminobenzaldehyde nitrite	Tryptophan	−
Potassium permanganate/alcian blue	Disulfides	+
Ferric ferricyanide	Sulfhydryl groups	+ +
Ninhydrin/Schiff	Protein-bound NH_2 groups	+ + +
Feulgen reaction	DNA	+
Pyronin Y	RNA	+ +
Copper phthalocyanin	Phospholipids	+
Sudan black B	Lipids	+

+ + +, Intensely positive; + +, Strongly positive; +, Moderately positive; ±, Faintly positive; −, Negative.

is a small epithelial cell consistent with that of *C. annulata.* However, the second cell type is described as containing basophilic cytoplasmic granules and is much rarer in occurrence. It appears that this second cell type may be representative of granular haemocytes, which in *C. annulata* can be found within the haemal sinuses of the Y-organ. The haemal spaces may be obliterated or quite open in intermoult crabs. When these spaces are closed, the haemocytes present in them might easily be mistaken for cells pertaining to the gland proper. Simione and Hoffman[17] also expressed the same opinion regarding the presence of the second type of cell. Bressac[18] found that mitotic activity in the Y-organ of *Pachygrapsus marmoratus* began to rise in late C_4 and reached a peak in late D_1. Le Roux[5] found that nuclear size varies according to the position of the cell within the organ of *Palaemon serratus.* But in the present study neither mitotic activity nor nuclear size variation could be found.

So far, there has been no comprehensive study of the cytochemical nature of the Y-organ in crustaceans. The present study reveals that Y-organs are rich in proteins. Glycogen is absent but traces of 1:2 glycol groups and mucopolysaccharides are present. Gabe[1,2] also reported the absence of glycogen. Hinsch and al Hajj[19] reported the presence of glycogen in the Y-organ of *Libinia emarginata* which appears in part to be a reflection of season. Although Durand[15] reported aldehyde fuchsin positive secretory material in *O. limosa*, Matsumoto[20] could not find any secretory material in the crab *Hemigrapsus nudus.* No such stainable material is detected in the Y-organ of *C. annulata.* Large amounts of sulfhydryl groups, protein-bound amino groups and RNA-positive material are encountered. The pyrinophilic nature was also reported by Gabe[2]. According to Adiyodi and Adiyodi[14] electron micrographs of the Y-organ of intermoult *Pachygrapsus crassipes* show that the cytoplasm contains no formed secretory products, but several types of lysosomal inclusions which may perhaps account for the stainable substances observed by Gabe.

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